Rejection of Claim 32 under 35 U.S.C. §112, Second Paragraph

The Examiner has rejected Claim 32 under 35 U.S.C. §112, second paragraph, as being indefinite because it lacks antecedent basis for the limitation, "...cell is isogenic, allogenic or xenogenic."

Support for isogenic, allogenic and xenogenic cells is provided in the Specification, for example, on page 15, lines 17-21. Further, Claim 32 is dependent on Claim 26, which has been amended to include a "...cellular-based cardiac biological pacemaker comprising at least one cell...". Amended Claim 26 combined with the description in the Specification, therefore, provides proper antecedent basis for Claim 32, thereby obviating the rejection. Withdrawal of the rejection of Claim 32 under 35 U.S.C. §112, second paragraph is respectfully requested.

Rejection of Claims 33-71 under 35 U.S.C. §112, First Paragraph

The Examiner has rejected Claims 33-71 under 35 U.S.C. §112, first paragraph. According to the Examiner, the Specification, "...while being enabling for the expression of the β_2AR gene *in vitro* in myocardiac by transfection of the construct and being enabling for increase rate of contraction in the transduced cells, does not reasonably provide enablement for *in vivo* correction of cardiac dysfunction in mammals." Thus, the Examiner believes that "...the specification does not enable any person skilled in the art to which it pertains, or with which it is most closely connected, to use the invention commensurate in scope with these claims." (Page 3 of the Office Action).

Applicants have canceled Claims 34-42, 44, 46, 47, 49-51, 55, and 59-68.

Claims 33, 43, 45, 48, and 52-54, are dependent on Claim 26 or on Claims that are dependent on Claim 26, which has been amended to recite a gene that "...upregulates heart rate or alters cardiac rhythm." Claim56 is an independent Claim that was originally directed to a pacemaker that "...upregulates heart rate or alters cardiac rhythm." Claims 57 and 58 depend from Claim 56 and do not introduce further limitations directed to "...correction of cardiac dysfunction...". As amended, Claims are drawn to the regulation of heart rate or contractility of cells. Data enabling this aspect of the invention is disclosed in the "Examples" section of the Specification, and, further, as pointed out by the Examiner on page 7 of the Office Action, "[t]he specification describes β_2AR expression and increased [sic] in contractile rates in *in vitro*

cultured myocytes and β_2AR expression and increased heart rates *in vivo* heart or transplanted heart."

Applicants note that the Specification provides support for expression of the β_2AR gene in cultured cells, murine hearts and porcine hearts. As the cells are intact cells, and not extracts or reconstituted systems, the β_2AR gene is expressed *in vivo*. Although manipulation of the cells is an *in vitro* process, the cellular functions, *e.g.*, expression of a gene, are not typically referred to as "*in vitro*". Rather, as defined in exemplary textbooks, "*in vivo*" refers to conditions in "an intact cell or organism" (see, for example, Lodish, H. *et al.*, Molecular Cell Biology, pg. G-10; attached herewith as Exhibit I). In fact, as described in the present Application, Applicants have "...provide[d] a combination of *in vitro*, *ex vivo*, and *in vivo* gene transfer techniques useful for the...identification and characterization of genes that could be employed to selectively upregulate heart rate and alter cardiac rhythm in an intact heart" (page 9, lines 25-28 of the Application; see also, the Exemplification).

More to the point, the Examiner's rejection concerns the relationship between expression in cultured cells and expression in an organism. The Examiner cites several references teaching the unpredictability of gene therapy (see below) Applicants submit, however, that the claimed invention does not encompass the gene therapy methods as described in the Office Action. Applicants further submit two methods for expression, cell grafting and direct injection, are methods that, both in the Specification and the art (see Alexander *et al.*), predictably allow for expression of genes that upregulate heart rate or alter cardiac rhythm.

The Examiner cites Verma and Somia (Nature, 1997, 389:239-242) as teaching that gene therapy *in vivo* continues to be unpredictable and inefficient. However, Applicants claimed invention, as amended, is not limited to the use of targeted gene therapy vectors. Applicants' claimed invention has been amended to claim expression by either direct injection of plasmid DNA into the myocardium or by cell grafting ("cellular transplantation"). Targeted gene expression obtained using either of these expression methods has been reported (Alexander *et al.*). Reports in the art combined with the Specification, thus, eliminates the unpredictability of targeted gene expression inherent in the vector-based delivery methods described by Verma and Somia. Although direct plasmid injection is mentioned in the teachings of Verma and Somia, the teachings of Alexander *et al.* show that the expression of naked DNA that is directly injected into

the myocardium is possible. This teaching appears to be "...unique for striated muscle and does not occur in other organs at significant levels" (Alexander *et al.*, pg 662). Thus, Applicants submit that for the scope of the claimed invention, gene expression in the myocardium by either direct injection or by cellular transplantation was known in the art at the time of filing.

The Examiner cites Hajjar *et al.* (*Circ. Res.*, 2000, 86:616-621), as teaching "...it is important to acknowledge that the field of gene therapy has yet not proven its clinical value in any context," and Alexander *et al.* (*Clin. Exp. Pharm. Phys.*, 1999, 26:661-668), as teaching the unpredictability of gene therapy ("Cellular transplantation may hold promise for repair of compromised myocardial tissue, but its application to gene therapy approaches must await the demonstration of a functional improvement in the myocardium following cell grafting" (pg. 666, paragraph 4)). Applicants point out that the Claims are directed to a cardiac pacemaker composition and methods for using the composition to upregulate heart rates or alter cardiac rhythm. Specific clinical improvements are not specified in the Claims. Applicants submit that the Specification provides support for increased chronotropic function, and, as such, provides support for the functioning of the disclosed invention as a cardiac pacemaker.

The Examiner further cites Alexander *et al.* as teaching that grafted myoblasts can be successfully transplanted into myocardium with no sign of tissue rejection. Independent Claims 26 and 56, and all Claims dependent on Claims 26 or 56, recite cardiac pacemaker cells that would presumably be relevant to the teachings of Alexander *et al.* The Examiner argues that the teachings of Alexander *et al.* show that cell grafting does not lead to increased heart rate, as, in Alexander *et al.*, no changes in myocardial function were observed. However, Applicants respectfully submit that the myoblasts that were transplanted were normal skeletal myoblasts, and, thus, did not overexpress pacemaker genes, as provided by Applicants' claimed invention. Applicants' claimed invention is directed, in part, to cells overexpressing genes that regulate cardiac chronotropy, *e.g.*, βAR's and G_{as}. In fact, Applicants submit, the fact that Applicants are able to demonstrate cardiac pacemaker activity where the prior art failed, is a result of the expression of these chronotropy regulatory genes. As such, the demonstrated cardiac pacemaker activity of Applicants' claimed invention represents a significant and unexpected result over the

art on this point. The combination of the art demonstrating that cellular transplantation allows for gene expression without tissue rejection and the Specification of the application demonstrating a cardiac pacemaker activity, Applicants submit, sufficiently enable one of skill in the art to practice all aspects of the present invention directed to a cardiac pacemaker that includes cellular transplantation.

As stated above, direct DNA injection into myocardium has been demonstrated to lead to gene expression (Alexander *et al.*). In fact, the myocardium (and striated muscle more generally) is ideally suited to gene expression via direct DNA injection. Although Alexander *et al.* mention an inefficient expression, the description is relative to viral vectors that have been designed to maximize expression. Applicants submit the point is moot, however, in light of Applicants demonstration that direct DNA injection of a β_2AR expression vector is sufficient to provide cardiac pacemaker activity. This demonstration occurred *in vivo* in both murine and porcine models. The expression data in large animal models, *e.g.*, the Yorkshire pig, as thoroughly provided in the Specification, clearly describes a functioning example of Applicants' claimed invention.

Although the Examiner objects to the use of animal models as providing evidence to enable a skilled artisan to practice the present invention, Applicants submit that animal models are routinely taken as being indicative of likely success in humans or other animals. Indeed, as Applicants state, "The Yorkshire pig was chosen for its anatomic and physiologic similarity to the human cardiovascular system..." (page 27). Moreover, Alexander *et al.*, state that while "...many cardiac studies have been carried out in small animal models that may be informative in terms of cardiac function and ventricular performance under various inotropic and loading conditions, it is important to use large animal models with physiology similar to that of a human..." (pg 665). It is clear from the fact that subsequent examples described by Alexander *et al.*, that the authors are alluding to porcine models as being "large animal models" that are taken to be standard model animals. The courts have also consistently held that specific *in vivo* testing in humans is not required to satisfy the requirements of 35 U.S.C. §112 (1), as "...proof of an alleged pharmaceutical property for a compound by statistically significant tests with standard experimental animals is sufficient to establish utility" (*In re* Brana, 51 F.2d 1560, 1567).

Applicants have thus satisfied this criterion deemed important by representative art and the courts prior to the time of filing.

The Examiner also cites Drazner *et al.* (*Proc. Assoc. Am. Phys.*, 1997, 109:220-227) as teaching, "[i]t is not possible to know the full consequences of cardiac overexpression of specific components of βAR signaling cascade *a priori*. Because the overexpression of β_2ARs, $G_{\alpha s}$ or a βARK inhibitor can enhance cardiac function beyond the normal physiological state, it is likely that such strategies could improve the inotropy of a failing myocardium. However, as these three strategies have fundamentally different mechanisms of action, it is possible that they may lead to different hemodynamic consequences and outcomes in survival when applied to animal models of CHF." In response, Applicants submit that evidence is provided in the Specification and specific Examples are disclosed that describe the effect of the overexpression of the βAR signaling cascade *in vitro* (Example 1), *ex vivo* (Examples 2 and 3) and *in vivo* (Examples 3 and 4). As described above, Applicants have provided data obtained, in addition to cultured cells, in murine and porcine models that allow for, as recognized by the art at the time, a reasonable likelihood of success in human tissues. The courts have consistently ruled that a Specification is enabling as long is it reasonably directs a worker of ordinary skill in the art to perform routine experimentation, so long as such experimentation is not unreasonable (*In re Wands*, 858 F.2d 731). Thus, Applicants have data, and do not rely on erroneous *a priori* assumptions, to substantiate the functioning of the cardiac pacemaker as it appears in the Claims.

Rejection of Claims 26, 28, 29-31 and 43 under 35 U.S.C. §102

The Examiner has rejected Claims 26, 28, 29, 31 and 43 under 35 U.S.C. §102(b) as being anticipated by Milano, *et al.*, (Science, 1994, 264:582-586).

The Examiner points out that Milano *et al.* teach a construct that allows for the stable expression of β_2AR in murine heart. Applicants submit, however, that Milano *et al.* teach the expression of β_2AR in transgenic mice and would not be enabled to use such a construct for the methods described in Applicants' amended Claims. Milano *et al.* do not teach the use of a construct in a cellular-based cardiac pacemaker, nor do they teach the introduction of a construct

by direct injection. Therefore, the teachings of Milano *et al.* do not anticipate Applicants' claimed invention as such constructs are not within the scope of the claimed invention.

The Examiner has rejected Claims 26, 28-31 and 43 under 35 U.S.C. §102(b) as being anticipated by Gaudin, *et al.*, (J. Clin. Invest., 1995, 95:1676-1683).

The Examiner points out that Gaudin *et al.* teach a construct that allows for the stable expression of $G_{\alpha s}$ in cardiac tissue. Applicants submit, however, that Gaudin *et al.* teach the expression of $G_{\alpha s}$ in transgenic mice and would not be enabled to use such a construct for the methods described in Applicants' amended Claims. Gaudin *et al.* do not teach the use of a construct in a cellular-based cardiac pacemaker, nor do they teach the introduction of a construct by direct injection. Therefore, the teachings of Gaudin *et al.* do not anticipate Applicants' claimed invention as such constructs are not within the scope of the claimed invention.

The Examiner has rejected Claims 26, 28-31 and 43 under 35 U.S.C. §102(a) as being anticipated by Drazner, *et al.*, (*J. Clin. Invest.*, 1997, 99:288-296).

The Examiner points out that Drazner, *et al.*, teach transduction of cultured myoblasts by an adenovirus construct comprising β_2AR. Drazner *et al.*, however, do not teach regulating cardiac pacemaking activity, upregulation of heart rate or altered cardiac rhythm. Drazner *et al.* teaches the effects of β_2AR overexpression on particular cell-signaling and ligand-binding events in cultured cells. The teachings of Drazner *et al.*, only demonstrate a potentiation of βAR signaling and does not provide a teaching of cardiac pacemaker activity. Additionally, the teachings of Drazner *et al.* do not provide methods for targeted expression of the adenovirus vector expressing β_2AR, *e.g.*, to a particular tissue type. As such, the teachings of Drazner *et al.* do not describe features of Applicants claimed invention, and, therefore, can not anticipate Applicants claimed invention.

CONCLUSION

In view of the above amendments and remarks, it is believed that all claims are in condition for allowance, and it is respectfully requested that the application be passed to issue. If the Examiner feels that a telephone conference would expedite prosecution of this case, the Examiner is invited to call the undersigned at (781) 861-6240.

Respectfully submitted,

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MARKED UP VERSION OF AMENDMENTS

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

26. (Amended) A cellular-based[molecularly-mediated] cardiac biological pacemaker [construct] comprising at least one cell transfected or transduced with at least one gene that upregulates heart rate or alters cardiac rhythm suitable for localized gene expression in mammalian cardiac atrial tissue.

28. (Amended) The cardiac pacemaker of Claim 26[27] wherein the gene is selected from the group consisting of: a β_2AR gene, β_1AR gene, and $G_{\alpha s}$ gene.

29. (Amended) The cardiac pacemaker of Claim 28 wherein gene expression is regulated by at least one [the construct further comprises] expression control element[s].

32. (Amended) The cardiac pacemaker of Claim 30[31] further wherein the cell is isogenic, allogenic, or xenogenic.

33. (Amended) A method of regulating cardiac pacemaking activity in a mammal by introducing a biologic pacemaker according to Claim[s] 26[7] into the sinoatrial node region of a mammalian heart.

48. (Amended) A method of regulating [improving] cardiac pacemaking activity [function] in a mammal [senescent heart tissue] by introducing a biological pacemaker according to Claim 45 into an atrial chamber of a mammalian heart.

52. (Amended) The method of Claim 33[48] wherein the biological pacemaker is a cellular-based cardiac pacemaker construct comprising a transfected or transduced cell expressing a β2-adrenergic receptor and further wherein the method comprises *in vivo* administration of an adrenergic agonist.

58. (Amended) A method of regulating cardiac pacemaking activity in a mammal by introducing a biologic pacemaker according to Claim 56[31] into the sinoatrial node region of a mammalian heart.

70. (Amended) A method of [permanently] regulating cardiac pacemaking activity in a mammal by introducing a molecularly-mediated cardiac pacemaker construct comprising at least one gene that upregulates heart rate or alters cardiac rhythm suitable for localized stable gene expression in mammalian cardiac atrial tissue, wherein said construct is introduced by direct myocardial injection or endocardiac transfection or transduction.

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